Qualitative immunoassay for detection of Antibodies to HIV 1/ 2 in Oral Mucosal Transudate -A Diagnostic Study

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CERTIFICATE

Certified that the dissertation on , **"Qualitative immunoassay for detection of Antibodies to HIV 1/ 2 in Oral Mucosal Transudate -A Diagnostic Study"** done by Dr.J.Suhanya, Post Graduate student (M.D.S), **Branch IX Oral Medicine and Radiology**, Tamilnadu Government Dental College and Hospital, Chennai – 600 003, submitted to The Tamilnadu Dr. M.G.R Medical University for partial fulfillment of the M.D.S degree examination in March 2010, is a bonafide research work done under my guidance and supervision.

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DECLARATION

TITLE OF THE DISSERTATION	**Qualitative Immunoassay for Detection of antibodies to HIV 1/2 in Oral Mucosal Transudate- A Diagnostic Study**
PLACE OF STUDY	**Tamilnadu Government Dental College and Hospital, Chennai**
DURATION OF THE COURSE	**3 Years**
NAME OF THE GUIDE	**Dr.S.Jayachandran, MDS**
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Guide and Head of the Department Signature of the Candidate

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CONTENTS

Qualitative Immunoassay for detection of Antibodies to HIV 1/2 in Oral Mucosal Transudate - A Diagnostic Study

Introduction

Acquired Immuno Deficiency syndrome is a fatal disease caused by a Retro virus called **Human Immuno Deficiency Virus** which is a RNA virus and is characterized by profound Immunosuppression together with development of opportunistic infections, Secondary Neoplasm's and neurologic manifestations[5].

Centers of Disease control modified AIDS definition[6] in 1992 as "All people infected with HIV who have a CD4 lymphocyte count of < 200 cells per micro liters irrespective of clinical manifestations / who have one of the following 3 clinical conditions, Pulmonary tuberculosis , Recurrent pneumonia and Invasive cervical cancer".

WHO[6] defined AIDS by the existence of atleast two of the major signs associated with atleast one minor sign in the absence of known case of immunosuppression such as cancer / severe malnutrition. Major Signs include Weight loss > 10% body weight, chronic diarrhoea > 1 month, prolonged fever > 1 month. Minor signs include persistent cough for > 1 month, generalized pruritis dermatitis, recurrent herpes zoster,

Oropharyngeal candidiasis, chronic progressive / disseminated herpes simplex infections, and generalized lymphadenopathy.

Epidemiology:

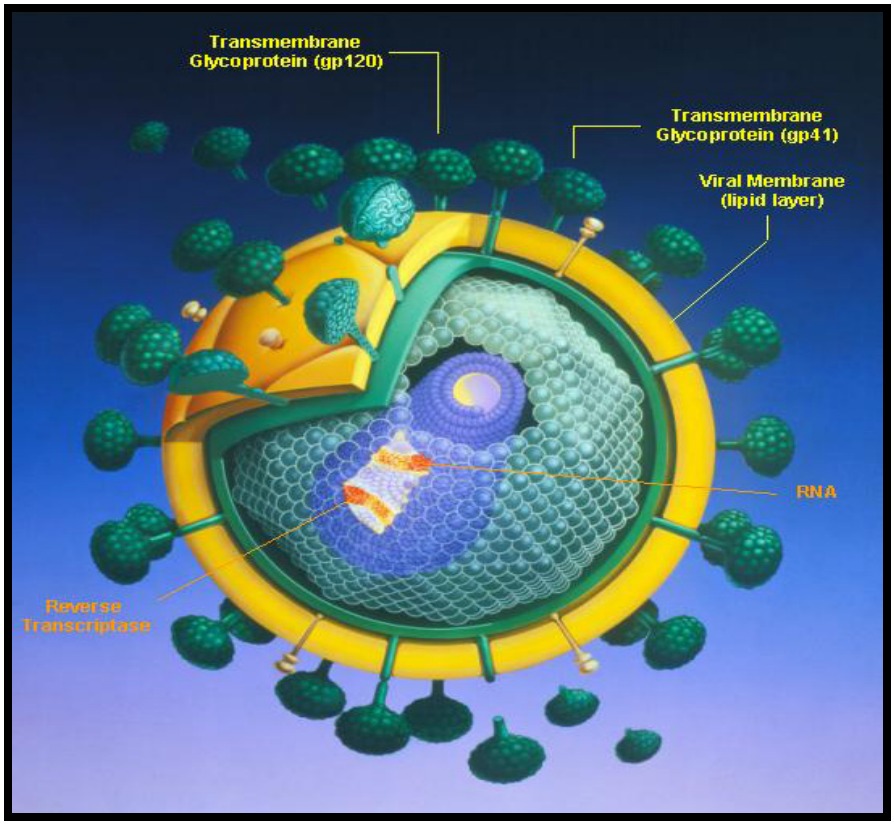
HIV infection /AIDS is a global pandemic. The current estimate of the number of cases of HIV infection among adults worldwide is approximately 37 million, two thirds of whom are in sub- Saharan Africa. 50% cases are Women[5]. In addition 2.5 million children younger than 15 are living with HIV/AIDS. According to Joint United nations Programme on HIV/AIDS[1] in 2007, on a global scale, the HIV epidemic has stabilized, although with unacceptably high levels of new HIV infections and AIDS deaths. Globally, there were an estimated 33 million [30 million–36 million] people living with HIV in 2007[11]. The annual number of new HIV infections declined from 3.0 million [2.6 million- 3.5 million] in 2001 to 2.7 million [2.2 million–3.2 million] in 2007.Overall, 2.0 million [1.8 million–2.3 million] people died due to AIDS in 2007, compared with an estimated 1.7 million [1.5 million–2.3 million] in 2001[6]. India had an estimated 1.8 – 2.9 million HIV positive persons in 2007, with an estimated adult HIV prevalence of 0.34% (0.25%–0.43%)[2]. The most rapid and well-documented spread of infection has occurred in State of Maharashtra (Bombay) and Tamilnadu. The HIV epidemic has occurred in waves in different regions of the world, each wave representing different characteristics depending on the demographics of

the country and region in question and the timing of the introduction of HIV into the population.

Virology:

Human Immuno Deficiency Virus type 1 and type 2, a RNA virus belongs to the Lentivirus a subfamily of Human Retrovirs[6-9]. HIV 1 and HIV 2 is the only Lenti Virus known to infect humans. The hallmark of the HIV virus is the reverse transcription of its genomic RNA to DNA by the enzyme reverse transcriptase. Electron Microscopy shows that the HIV virion is an icosahedral structure containing numerous external spikes formed by the two major envelope proteins, the external gp 120 and the transmemebrane gp 41. HIV type 1 and HIV type 2 are two subtypes of HIV which is associated with AIDS. Same Individuals can be infected with both HIV-1 and HIV-2. HIV-1 is more aggressive[3] and more transmissible than HIV -2 .The transmission efficiency of HIV-1 appears considerably greater than HIV-2 early in course of infection, which account for more rapid spread of HIV-1. There is a higher titre of autolog of neutralizing antibodies in HIV-2 infected individuals .These antibodies survive for a longer time. Various strains of HIV type 1 and HIV type 2 exists and have some significant nucleic acid and envelope differences. These variants of HIV strains promote a rapid diseases progression.

HIV Virus Structure



Pathogenesis:

HIV primarily affects cell-mediated immunity binding to and infecting cells that have CD4 surface antigen. CD4 is found on T-helper cells, macrophages, monocytes, and colorectal cells possibly some brain cells. Disease progression is the result of HIV affinity for T4 cells which promote activity of cell mediated immune response primarily located in lymphoid tissue[6-9]. HIV binds to the CD4+Tcell via a specific high affinity interaction of the external envelope glycoprotein, gp120. HIV fuses with the cell using transmemebrane gp41[6]. Major co-receptors

CCR5 and CXCR4 are required for fusion and entry into the cell. After fusion HIV genome is uncoated and internalized into the cell. Viral Reverse transcriptase mediates transcription of its RNA into double stranded DNA which is integrated into the genome of the infected cell through the action of the viral enzyme integrase, causing a latent infection. From time to time, lytic infection is initiated releasing progeny virions which infect other cells. The long and variable incubation period of HIV infection is because of latency. As a result T4 cells decrease in numbers, T4: T8 cell ratio is reversed[12]. Infected T cells do not appear to release normal amounts of interleukin-2, gamma interferon, and other lymphokines. This has a marked damping effect on cell mediated immune response. Helper T cell activity is essential for optimal B cell function humoral mechanisms are also affected. Monocyte-macrophage function is also affected apparently due to lack of secretion of activating factors by the T4 lymphocytes. As a result of chemotaxis, antigen presentation and intracellular killing by monocytes / macrophages are diminished. The activity of NK cells and cytotoxic T lymphocytes is also affected[9]. Clinical manifestations of HIV infections are not primarily due to cytopathology but are secondary to the failure of immune responses and subsequently opportunistic infections[6-9].

Mode of transmission / High Risk Factors:

The Three Main Routes of Transmission are Sexual Contact, Intravenous drug abuse and perinatal exposure[5-9]. Infection may also occur from artificial insemination, organ transplantation and breast feeding. High Risk group include IV drug user using contaminated needles, homosexual and bisexual men and infants of HIV positive mothers[3]. Recently there has been a serious increase of HIV Positive women within heterosexual population. Unprotected receptive and anal intercourse is highest risk behaviour by far among homosexual. High risk sexual behaviour is increased in case of multiple partners and in case with sexually transmitted disease. There is no evidence HIV transmitted through contact with air environmental surface, casual contact even where people are living together. HIV may be found in variable numbers at various times during the course of disease in the lymph nodes , nervous system , blood , serum, semen, urine, breast milk , saliva , tears and vaginal fluid . HIV does not survive for long outside[5].

Oral Manifestations of AIDS:

Characteristic Oral infection represents earliest manifestations of HIV infection. Some lesions may be of prognostic significant in development of AIDS. Some produces very serious discomfort and morbidity. Specific Oral lesions and concurrent different oral lesions are

indicators of severe immunosuppression. The predictive values of lesions indicating severe suppression ranked with the highest are Major Apthous Ulcer, Necrotic Periodontitis, Kaposi's Sarcoma, Long standing herpes simplex infection , Hairy leukoplakia and Xerostomia. Oral hairy Leukoplakia and Oral candidiasis are the most common and thus best prognostic indicators of the development of AIDS[5].

EC- Clearinghouse Classification of the Oral Manifestations of HIV diseases:[66] (J Oral Pathol Med 1993; 22: 289-91, J Oral Pathol Med.2005; 34: 513-31)

Group 1 lesions strongly associated with HIV infection:

- Candidiasis
  - Erythematous
  - Pseudomemebranous
- Oral Hairy Leukoplakia
- Kaposi's sarcoma
- Non Hodgkin's Lymphoma
- Periodontal Diseases
  - Linear Gingival Erythema
  - Necrotizing gingivitis
  - Necrotizing Periodontitis

Group 2 lesions less commonly associated with HIV infection:

- Bacterial infections
  - Mycobacterium Avium-Intracellulare
  - Mycobacterium tuberculosis
- Melanotic hyperpigmentation
- Necrotizing Stomatitis
- Salivary gland diseases
  - Xerostomia
  - Unilateral or bilateral swelling of major salivary glands
- Thrombocytopenic Purpura
- Ulceration not otherwise specified

Viral infections
- Herpes simplex Virus
- Human Papillomavirus lesions
- Condyloma Acuminatum
- Focal epithelial hyperplasia
- Verruca Vulgaris
- Varicella Zoster Virus
- Herpes Zoster Varicella

Group 3 lesions seen in HIV infection:

Bacterial infections
- Actinomyces Israeli
- Escherichia Coli
- Klebsiella Pneumonia

Cat –Scratch diseases

Drug –reactions
- Ulcerative
- Erythema Multiforme
- Lichenoid reaction
- Toxic epidermolysis
- Epitheliod bacillary angiomatosis

Fungal infections other than Candida
- Cryptococcus neoformans
- Geotrichum candium
- Histoplasma capsulatum
- Mucormycosis
- Zygomycosis
- Aspergillus flavus

Neurological disturbances
- Facial palsy
- Trigeminal Neuralgia
- Viral Infections
  - Cytomegalovirus
  - Molluscum Contagiosum

EC- Clearinghouse and WHO classification of Oral Manifestations of pediatric HIV disease[66]:

Group 1 lesions commonly associated with pediatric HIV infection

- Candidiasis
  - Erythematous candidiasis
  - Pseudo membranous candidiasis
  - Angular Chelitis
- Herpes simplex virus infection
- Linear gingival erythema
- Parotid enlargement
- Recurrent apthous ulcers
  - Minor
  - Major
  - Herpetiform

Group 2 lesions less commonly associated with pediatric HIV infection

- Seborrheic dermatitis
- Bacterial infection of oral tissues
  - Necrotizing ulcerative stomatitis
- Periodontal diseases
  - Necrotizing (ulcerative) gingivitis
  - Necrotizing (ulcerative) periodontitis
- Viral infections
  - Cytomegalovirus
  - Human papilloma virus
  - Varicella –Zoster virus
    - Herpes Zoster
    - Varicella
- Xerostomia

Group 3 lesions strongly associated with HIV infection but rare in children

- Neoplasm
  - Kaposi's sarcoma
  - Non-Hodgkin's lymphoma
- Oral hairy leukoplakia
- Tuberculosis –related ulcers

Laboratory Tests:

Laboratory diagnosis for diagnosis of HIV includes specific tests for HIV as well as tests for immunodeficiency[7-9].

A. Immunological tests

B. Specific Test for HIV infection

A. The Immunodeficiency of the individual can be established by the following parameters.

   a. Total Leukocyte / Lymphocyte count

   b. T cell subset assay: Absolute CD4 & Tcell Count, T4: T8 ratio.

   c. Platelet Count

   d. Raised IgG / IgA level

   e. Lymph node biopsy

B. Specific Tests for HIV infection:

The specific test for detecting HIV infection depends on demonstration of HIV antigens and its components, antibodies, isolation of Virus.

Antigen detection:

The Viral antigens may be detectable in blood after about two weeks of HIV infection. The major Core antigen P24 is the earliest viral marker to appear in blood. Free P24 disappears from circulation and

remain absent during long asymptomatic phase, but antibody bound P24 antigen can be demonstrated. P24 capture assay which use anti P24 antibody is positive in 30% of HIV individuals the test is used to diagnose patients recently exposed to HIV infection where antibody test is negative[7-9].

Viral Isolation:

Viral Isolation is not suitable as a routine diagnostic test. The Risk involved Virus isolation is to be considered and has to be carried out only in laboratories with adequate facilities. The Technique of Viral isolation is by Co-cultivation of the patients lymphocytes with uninfected lymphocytes in the presence of interleukin -2. Viral replication can be demonstrated by Reverse transcriptase activity as well as by the antigens[9].

Polymerase Chain Reaction:

Polymerase chain reaction has become the gold standard for diagnosis of all stages of HIV infection. The PCR tests are complex and costly and are indicated only when other methods cannot give a definitive result[9].

Antibody Detection:

The antibody test which is current in practice is ELISA, Rapid serum tests, Rapid Salivary and Urine assays and Western Blot[6].

ELISA:

ELISA is a highly specific screening test to test HIV antibody. Direct solid phase antiglobulin ELISA is the method most commonly used. The antigen is obtained from HIV grown in continuous T lymphocytes cell line or by recombinant techniques and should represent all groups and subtypes of HIV-1 and HIv-2. The antigen is coated on micro titre wells or other suitable solid surface. The test serum is added and if the antibody is present, it binds to the antigen. After washing away the unbound serum, antihuman immunoglobulin linked to a suitable enzyme is added, followed by a color –forming substrate. If test contains anti – HIV antibody, a photo metrically detectable color is formed which can be read by special ELISA readers. ELISA is simple and relatively inexpensive but false positive reactions are not uncommon, while ELISA is ideal for screening several serum samples at a time, it is inconvenient for testing single samples quickly. A number of ‘ rapid tests’ have been introduced for this purpose such as cylinder or cassette ELISA, immunochromatographic, coated particle agglutination, immunoperoxidase or dip stick tests. Tests using finger-Prick blood, saliva and urine have also been developed[7-9].

If ELISA is positive, a confirmatory Western blot test is performed

Western Blot:

Western Blot is considered 'Gold Standard' confirmatory tests. Identifies antibodies to HIV proteins and glycoprotein and a positive test provides evidence of previous HIV exposure. In this test HIV proteins separated according to electrophoretic mobility by polyacrylamide gel electrophoresis are blotted onto strips of nitrocellulose paper. These tests are reacted with test sera and then with enzyme conjugated antihuman globulin. A suitable substrate is added which produces color band where the specific antibody has reacted with the separate viral protein. The position of the band on the strip indicates the antigen with which the antibodies have reacted with the separate viral proteins. In a positive serum bands will be seen with multiple proteins, typically with P24 (gag gene), P31 (pol gene) and gp41, gp120, gp160 (env gene). A positive reaction with proteins representing three genes gag, pol, and env is conclusive of HIV infection. Western blot is a very useful confirmatory test but the interpretation remains subjective and demands considerable experience. If no definitive result can be given then, it may be necessary to have p24 assay done.

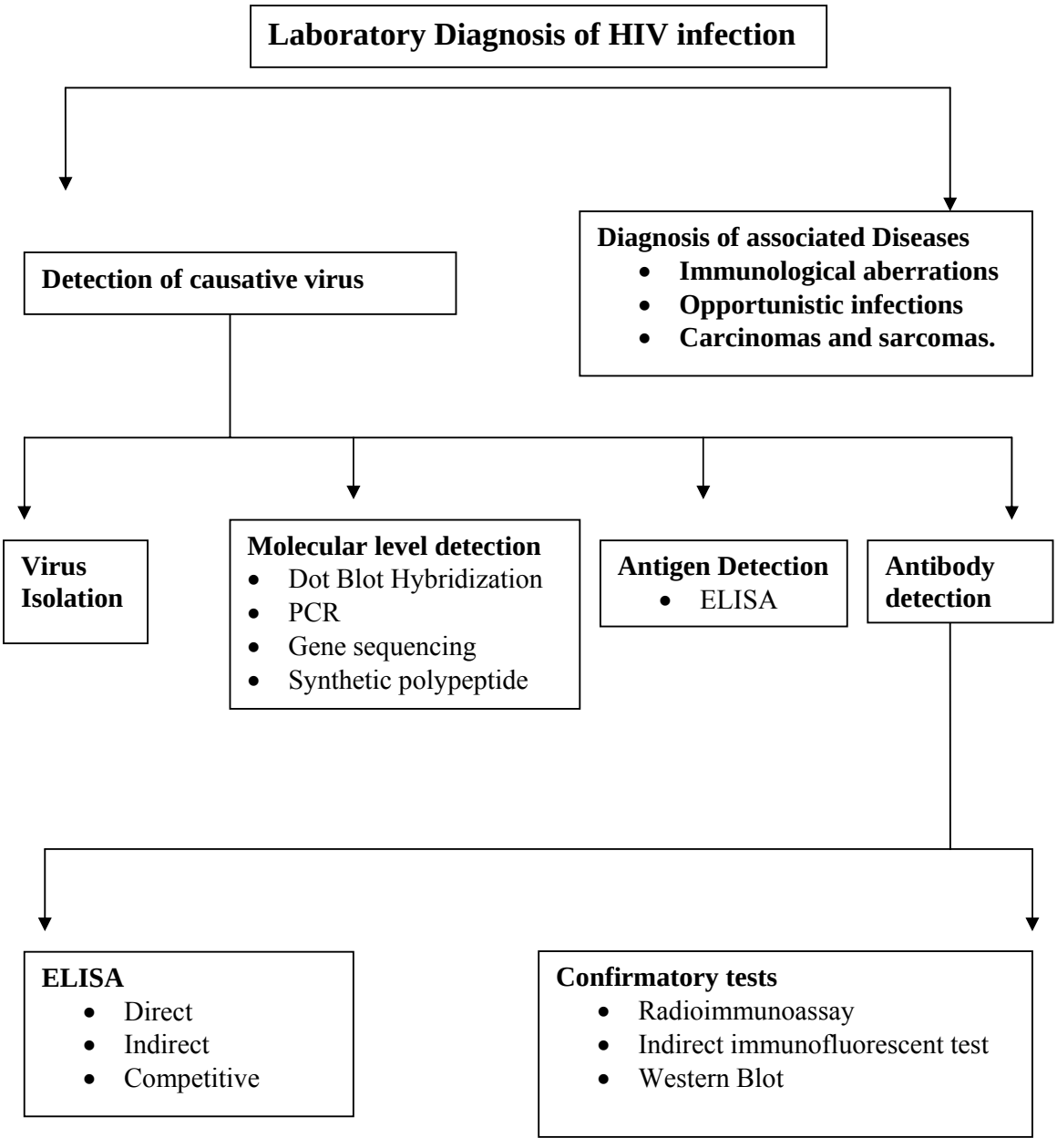
However since the test is costly, the practice now is to perform either two different types of ELISA or an ELISA with any of the rapid tests. A serum positive in both tests is considered positive. When in doubt retesting after 1 or 2 months may be useful. However serological

diagnosis of infections may not always be reliable in aids as antibody formation may be affected by the immune deficiency.

Oral Tests for HIV antibody[9]:

Various Oral Tests to detect HIV antibody either in Whole saliva or Oral Mucosal Transudate have become popular in recent years though it have been introduced long before. The possibility that saliva and oral fluids could be used for HIV screening and Diagnosis of HIV infections have been known since 1986. Oral Screening tests for HIV uses either IgA antibody of saliva or IgG antibody of Oral fluids. The advantages over serum screening test are ease of collection, safety, compliance and cost. Antibodies to Human Immuno deficiency virus type 1 have been found in various body fluids such as saliva, breast milk, cervical secretions and semen. The advantage of rapid HIV tests particularly with oral fluid specimens, include increased availability of testing among populations at high risk for HIV infection and increased receipt of test results among those tested

Laboratory Diagnosis of HIV Infection- Flow Chart[9]



Aims and Objectives

Aims:

This Study was designed to evaluate "HIV 1/ 2 OMT Test", a new simple Oral Mucosal Screening test for Human Immunodeficiency Virus Type 1(HIV -1) and Human Immunodeficiency Virus Type 2 (HIV -2) & to compare its Sensitivity and Specificity with Serum Screening assays like ELISA and Rapid Serum assay.

Objectives:

The main Objective of this Study is Qualitative analysis of antibodies to HIV-1 and HIV-2 in Oral Mucosal Transudate of Known HIV Positive Individuals & clinically suspected cases of HIV infection. Also Oral Screening antibody tests are compared with Serum Screening antibody tests to assess the accuracy, reliability, specificity and sensitivity of oral tests.

- Qualitative detection of HIV antibodies in Oral mucosal transudate of Known HIV individuals.
- Qualitative Detection of HIV antibodies in Oral Mucosal transudate of clinically suspected cases of HIV infection and comparison with serum screening assays.

Review of Literature

Virology, Immunopathogenesis & Transmission of HIV:

Nicholas G. Mosca, Alicia Rose Hathorn (2006)[65] discussed the mucosal resistance to HIV. The studies of Protective anti HIV-1 immune response in mucosal tissues such as oral cavity are in progress. The first line of defense against mucosal HIV infections begins with virus neutralizing immunoglobulin or antibodies. In the oral cavity, secretory IgA produced by sub epithelial plasma cells is trancytosed by epithelial cells. IgG is transferred from blood by passive diffusion through gingival sulcus.

Jennifer L.Cleveland (2003)[71] evaluates the risk of occupational exposure of HIV to dental health care professionals (DHCP). Information available from the centers of Disease Control and Prevention (CDC) indicates that the risk of transmitting HIV to DHCP remains very small. Prospective studies worldwide indicate that the average risk of HIV infection after a single percutaneous exposure to HIV infected blood is 0.3%; after an exposure of mucous membrane in the eye , nose , or mouth approximately 0.1%. The precise risk of transmission after skin exposures is not known but is believed to be even smaller. Several factors affect the risk of HIV transmission after an occupational exposure. Laboratory studies have found that if needles that pass through latex gloves are solid

rather than hollow –bore or are of small gauge, they transfer less blood. In a retrospective case control study of health care professionals, an increased risk for HIV infection was associated with exposures to a relatively large volume of blood. The risk also was increased if the exposure was to blood from patients with terminal illness, possibly reflecting the higher titre of HIV in late- stage AIDS. Standard methods to prevent occupational exposure in dental settings include hand washing, use of barrier precautions and careful handling and disposal of sharp instruments.

Lauren L.Ptton (2003)[70] state that primary route of transmission of HIV are sexual, blood-borne and vertical. The oral cavity represents a unique site for potential mucosal transmission. Epidemiological evidence however suggests that oral transmission is rare despite detectable virus in saliva and oral mucosal cells of infected persons. Several endogenous mucosal antiviral factors such as virus- specific antibodies and soluble proteins are thought to play a critical role in preventing oral HIV transmission.

Eliaz Kaufman, Ira B. Lamster (2002)[10] Reviewed the diagnostic applications of Saliva for systemic diseases. As a diagnostic fluid, saliva offers distinctive advantage over serum because it can be collected non

invasively. Saliva also provides cost effective approach for the screening of large populations. Analysis of saliva may be useful in the diagnosis of hereditary disorders, autoimmune diseases, malignant and infectious diseases and endocrine disorders as well as in the assessment of therapeutic levels of drugs and monitoring of illicit drug use. They state that studies have demonstrated that the diagnosis of infection with the human immunodeficiency virus based on specific antibody in saliva is equivalent to serum in accuracy and therefore applicable for both clinical use and epidemiological surveillance. Antibody to HIV in whole saliva of infected individuals which was detected by ELISA and western blot assay correlated with serum antibody levels. As compared with serum sensitivity and specificity of antibody to HIV in saliva for detection of antibody to HIV in saliva are between 95% and 100%. Salivary IgA levels to HIV decline as infected patients become symptomatic. It was suggested that detection of IgA antibody to HIV in saliva may therefore be a prognostic indicator for the progression of HIV infection. Analysis of antibody in saliva is a simple diagnostic test for HIV compared with serum. Saliva can be collected non-invasively which eliminates the risk of infection for the health care workers. Furthermore viral transmission via saliva is unlikely since infectious virus is rarely isolated from the saliva. Several salivary and oral fluid tests have been developed for HIV diagnosis. Orasure is a testing system that is commercially available used

for diagnosis of HIV infection. The test relies on the collection of Oral mucosal transudate and IgG antibody. IgG antibody to the virus is the predominant type of anti- HIV immunoglobulin. Different pathologic lesions which are relatively common in HIV – infected individuals do not appear to influence the results. In conclusion, collection and analysis of saliva/ oral fluid offer a simple, safe well tolerable and accurate method for diagnosis of HIV infection.

Herenia.P.Lawrence (2002)[11] explores the recent research on salivary biomarkers of systemic illness, potential clinical and research applications of diagnostics based on oral fluids. Salivary biomarkers are used in monitoring general health and in the early diagnosis of disease. Disorders and diseases in which saliva may aid in diagnosis include human immunodeficiency virus seropositivity, cancer and preterm labor. Test using saliva as a diagnostic tool have made substantial inroads into an array of clinical and research areas such as virology, immunology, microbiology , endocrinology, epidemiology and forensics. The introduction of Polymerase chain reaction has lead to the use of oral fluids as a source of microbial DNA for detecting viruses and bacteria. In addition onset and severity of infectious diseases can be determined by the presence of antibodies to micro organism present in saliva and oral cavity. Researches have demonstrated that saliva tests for antibodies to

HIV represent a non-invasive alternative to quantification of antibodies in blood.

Laboratory diagnosis of HIV infection:

Stekler JD etal (2009)[14] state that antibody tests have longer window periods after HIV acquisition than do nucleic acid amplification tests (NAATs). They studied HIV antibody testing to homosexual men using OraQuick Advance Rapid HIV 1/2 antibody tests on oral fluid and finger stick blood specimens and compared with Enzyme Immunoassay. They concluded that rapid antibody tests are less sensitive than enzyme immunoassay during early HIV infection. NAAT should be integrated into HIV testing programs that serve populations that undergo frequent testing and that have high rates of HIV acquisition, particularly if rapid HIV antibody testing is employed. Antigen –antibody combination assays may be reasonably sensitive alternative to HIV NAAT.

Louie B (2009)[15] assessed the sensitivity and specificity of first, second, third generation EIA for detection of antibodies to HIV -1 in oral fluid. The performances of three blood-based immunoassays test kits were compared with regard their ability to detect HIV-1 antibody in oral fluid. It was found that these three kits differ in their ability to detect HIV-1 antibody. Notably, a third generation EIA which has been shown to possess superior sensitivity for antibody detection in plasma appears to

possess no sensitivity advantage for detecting HIV-1 antibody in oral fluid.

Anthony T.Vernillo etal (2007)[35] stated that latest Centres of Disease Control and Prevention (CDC) guidelines recommend routine HIV screening for a large segment of the population, given that the individual understands that HIV test will be performed unless he or she declines testing. Knowledge of HIV infection can increase early access to care and treatment and reduce further transmission. A rapid non-invasive test for HIV infection from oral fluid have recently gained importance and offers two distinct advantages 1) Results are available within 20 minutes, thereby eliminating a long waiting period 2) It has sensitivity and specificity comparable to blood testing. A preliminary positive test result must be confirmed with a Western Blot or by a standardized laboratory procedure. Important Ethical and legal issues must be resolved before the successful implementation of HIV testing in dental setting. The integration of HIV testing into dental practice is important and a policy of screening patient in dental office will contribute to a major advance in public health. Aside from legal and ethical considerations of HIV testing strategies and the education and training that would enable dentist to use salivary diagnostics for HIV infection, the implementation of and barriers to HIV testing in dental practice are of primary importance. HIV testing in dental settings will require that clinicians understand the legal and

moral requirements of privacy and confidentiality. A positive test result may lead to social ostracism, stigmatization in health care settings and employer discrimination. If a dentist administers the HIV test on oral transudate then he or she must give the preliminary results to the patient and then refer the patient to physician for follow-up confirmatory testing. Depending upon state law many clinics would be required to offer pre test and post test counseling. Rapid HIV testing in dental practice is now gaining momentum , suggesting that dentist must be willing to include such testing within their standard of health care delivery. Establishing new practices with rapid HIV testing in the dental setting will likely result initially from dentists currently in practice who wish to receive training in the use of the rapid test and counseling patients as part of their standard of care, positioning dentists at the leading edge of this important paradigm shift.

Roberts KJ etal (2007) reviewed the Outcomes of blood and oral fluid rapid HIV testing from 2000-2006 regarding four outcomes of rapid HIV testing: rates of client acceptance; rates of clients receiving their results; rates of entry into the medical care for those found to be HIV positive and the efficacy of prevention counselling after testing. A total f 116 studies in peer –reviewed journals were screened. Twenty six met the screening criteria and were selected for review. They stated considerable variation

was found in client acceptance rates with the highest rates among pregnant women in labor and delivery units and lowest rates in needle exchange and bath- house settings. Three studies on entry into medical care among those who were newly identified as positive were 47%, 82%, and 97% of clients adhering to first medical appointments. No long term medical follow up studies were available. Only one study evaluated the efficacy of prevention counseling after rapid testing and found no statistically significant differences in number of sexually transmitted diseases conventional versus rapid HIV testers contracted.

Delaney KP (2006)[32] evaluated the performance of a rapid HIV antibody test used with whole blood and oral fluid. In diverse settings in four studies, the OraQuick test showed high sensitivity and specificity for HIV antibody in whole blood and oral fluid specimens. Slightly more false-positive and false-negative results occurred with oral fluid than with whole blood, but performance with both specimen types was similar to, or better than, that of conventional EIAs

Gottfried TD (2006)[27] stated that the testing and counseling of persons at risk for infection with HIV and their subsequent treatment remains the primary tool to curb worldwide transmission of the virus. Rapid HIV tests address the need in the developing world for accurate, easy-to-use tests that do not require laboratory equipment or highly trained professionals

for implementation or refrigeration for storage. Calypte Biomedical has recently developed the Calypte AWARE HIV-1/2 OMT antibody test using oral fluid samples. This test has demonstrated high sensitivity and specificity for specimens collected in target areas where increased testing is needed. The inexpensive dipstick format in combination with the use of an alternative fluid to blood provides an improved testing procedure for areas with limited resources.

Wesolowski LG (2006)[23] did a post- marketing surveillance of Oral Quick whole blood and oral fluid rapid HIV testing. They state that Whole blood Ora Quick specificity was 99.98%, positive predictive value 99.24%, the median oral fluid specificity was 99.89% and positive predictive value was 90%.

Constantine NT, Kabat W (2005)[38] updated the laboratory diagnosis and monitoring of HIV infection. They stated that the simple and more accurate diagnostic tools have become available, particularly for early detection and to monitor treatment in those who receive anti-retroviral treatment. Laboratory tests range from simple antibody tests to more sophistical methods that are used to monitor disease progression and identify drug resistance. These tools can assist physicians, medical practitioners and laboratory personnel to select suitable diagnostic tools

for the diagnosis, screening, monitoring of disease progression and for detection of drug resistance to anti-retroviral therapies.

Lauren L.Ptton (2003)[70] state that standard serologic HIV antibody test methods consists of screening enzyme liked immunosorbent assay (ELISA) and confirmatory western blot tests. Technologic advances have resulted in the ability to easily and safely collect and test oral fluids for antibodies to HIV-1. In 2002, a rapid enzyme immunoassay HIV antibody antibody screening test that yields results in 20 minutes was approved by US FDA for use with blood. Clinical trials continue to improve this rapid test designed for use with oral mucosal transudate. Rapid test at the point of care facilities occupational-exposure post exposure prophylaxis decisions and may have value in identification of newly infected individuals. HIV testing requires written consent in most states and under most circumstances should be accompanied by pre-test and post-test prevention counseling according to CDC guidelines.

King SD etal (2000)[43] compared testing saliva and serum for detection of antibody to Human Immunodeficiency virus in Jamaica, West Indies. This study evaluated the OraScreen HIV Rapid Test, new simple saliva screening EIA for anti HIV1&2 and to compared the sensitivity and specificity with the standard serum anti HIV screening EIA in current use

in Jamaica. OraScreen HIV Rapid test showed 100% specificity and 100% sensitivity in 257 volunteers of a family planning clinic and 52 volunteers from a HIV infected individuals.

Sy FS Rhodes SD etal(1998)[45] studied the acceptability of Oral fluid testing for HIV antibodies in gay bars in a rural state and stated that oral fluid testing is acceptable and effective in reaching individuals who do not usually access traditional testing sites.

Sangare KA, Koffi AR (1997)[48] evaluated the potential of saliva for detection of antibodies and seroconversion in HIV individuals. They tested serum of 1023 patients with **Abbott recombinant HIV1/HIV 2 EIA** and the same samples with **Wellcozyme GACELISA** for saliva. They showed salivary ELSA test was 98.69% sensitive and 100% Specific. This Study shows that testing saliva is effective for determining HIV status early in seroconversion.

PJ. Lamey etal (1996)[49] collected various components of saliva, namely mixed saliva, crevicuar fluid and minor gland secretions were collected from 63 known HIV antibody seropositive patients. A commercial test system Wellcozyme HIV 1/2 and an antibody capture ELISA were compared for sensitivity against all components. Sensitivity of HIV 1/ 2

was 100% in 123 mixed saliva, 121 parotid saliva and 127 labial fluid samples and 98% in 99 submandibular samples and 127 crevicular fluid samples. Mixed saliva was most easily conveniently and effectively collected using a plain Salivette. According to this study the most effective sensitive method for demonstrating anti-HIV antibody is to collect mixed saliva with the plain Salivatte system and assay anti-HIV antibody levels by GACELISA.

Mortimer PP , Parry JV(1994)[52] state that salivary tests for anti-HIV offer advantages of convenience , economy and safety and are more acceptable to subjects than blood tests. Further evaluation of the collection devices and assays, the introduction of safeguards against inadequate sampling and the development of suitable confirmatory assays are required. When these deficiencies have been met, salivary tests may supercede tests on serum for HIV and also other infection.

Mortimer PP etal (1994)[52] stated that salivary tests for anti HIV offer advantage of convenience, economy and safety and are more acceptable to subjects than blood tests. Further evaluation of collection devices and assay, the introduction of safe guard against inadequate sampling and the development of suitable confirmatory assays are required. When these

deficiencies have met salivary test may supercede tests on serum for HIV and also other infections.

Shriniwas, Srivastava L (1994)[51] analyzed the various laboratory diagnosis of HIV infection states that Detection of HIV –specific antibodies can be done by using one or more screening procedures, ELISA, rapid tests and simple tests. Supplemental tests like Western blot, immunofluorescence assay (IFA), virus isolation may confirm a positive findings and provide additional information.

Matsuda S etal (1993)[54] studied the characteristics of IgA antibodies against HIV-1 in sera and saliva from HIV- seropositive individuals in different clinical stages and the results indicate that IgA antibodies are regulated independently from IgG antibodies and that the mucosal immune system is impaired in the early in the symptomatic phase of HIV infection. Detection of IgA antibodies may be useful in the prognosis of the diseases in HIV – infected individuals.

Klaus Stark etal (1993)[53] state that salivary antibody testing in saliva/oral fluid is an efficient tool for large scale epidemiological studies when standard salivettes are used for sample collection.

Archibald etal (1990)[61] in their study concluded that the presence of antibodies in saliva indicates that it is possible to stimulate production of secretory antibodies using vaccines against rgp 120. The development of

secretory antibodies may be necessary for protection against HIV infection. The presence of antibodies in saliva may inhibit the transmission of the virus across the mucosal membranes.

Oral manifestations:

Cristina Frezzini etal (2005)[66] reviews current trend of HIV disease of the mouth and stated that number of oral lesions presently not included in the EC Clearing house classification of Oral manifestations include : Exfoliative chelitis, eruptive chelitis associated with HAART, sub mucous fibrosis , brown hairy leukoplakia , petechiae oral erythema , ranula and chelitis glandularis. The HAART related oral lesions seem to be most commonly reported in individuals in the developed world. Oral candidiasis has been associated with a more frequent progression to AIDS and has been used as a clinical marker to define the severity of HIV infection. Pseudo membranous candidiasis is the most common clinical presentation. Resistance by HSV to acyclovir therapy can arise in HIV diseases although cidofovir proved to be clinically effective. Non Hodgkin's lymphoma is recognized as an AIDS defining condition in HIV infected individuals and is included in the oral lesions associated with HIV infection. Human papilloma virus has been increased in the mouth of HIV infected individuals in patients under HAART and is of aetiological significance in the development of oral squamous cell carcinoma. Highly active retroviral therapies generally reduce the

frequency and severity of most oral lesions associated with HIV diseases. The molecular epidemiological of the common oral infection is well characterized; however increased use of high dose of antimicrobials has lead to the development of drug resistance oral microbes.

Lauren L.Ptton (2003)[68] state that dentist may play a role in diagnosing HIV when medical history taking and oral examination reveal risk and clinical signs of possible immune suppression. Oral lesions, specifically intraoral Kaposi's sarcoma, oral candidiasis and oral hairy leukoplakia may be first clinical manifestations of HIV and have been shown to have relatively high positive predictive value for HIV infection.

Lauren L Patton etal (2000)[70] studied the changing prevalence of oral manifestations of Human Immunodeficiency virus in the era of protease inhibitor therapy. They have reduced the viral replication which often results in increase in CD4 Counts of more than 100 cells/mm^3. Parotid gland enlargement and Xerostomia associated with HIV –salivary gland disease appears to be on the rise. The observed increase in HIV salivary gland disease results from a similar lymphoproliferartive reactivation stimulated by HAART. Destructive immune mediated periodontal diseases, Oral Candidiasis and Kaposi's Syndrome appear to be on the

decline whereas HIV –Salivary gland diseases and oral warts are increasing significantly.

Anderdorf etal (1998)[72] studied the oral manifestations of HIV infection in 600 South African patients and concluded that one or more lesions are seen in 60.4% 0f cases. Combined candidal infections were evident in 37.8%, hairy leukoplakia 19.7% and combined gingival and periodontal lesion in 8.5%. Lesions less commonly recorded include oral ulcerations and Kaposi's sarcoma. The clinical range of lesions seen in similar to those reported elsewhere, but socio-cultural differences allowed no reliable comparison.

Greenspan.D, Greenspan JS (1993)[76] **state that** oral lesions represent some of the earliest signs of HIV infection and may be the prognostic significance in the subsequent development of AIDS and may if not treated produces significant morbidity. Oral examination is an important part of any physical examination and nowhere is this more important than in the case of suspected HIV infection. Oral candidiasis is a diagnostic monitor of AIDS in an HIV infected individual.

Materials and Methods

This Study was conducted in **Department of Oral Medicine and Radiology,** Tamilnadu Government Dental College and Hospital, Chennai -3; Institutional Ethical committee's approval had been got. Informed consent of each patient was obtained before conducting the study.

Duration of Study: Oct 2008-Oct 2009 (1yr)

Inclusion Criteria:

Sample Size:

Total Number of Patients: 50 Out of Which

25- Clinically Suspected Cases of HIV infection.

25- Known HIV Sero-Positive Individuals.

Age Group:

All patients of age 20- 60yrs of age male/ female were included in the study. Informed Consent of all the patients was obtained. All Subjects should be thoroughly examined which include medical history, contact history, a physical examination and clinical oral examination. HIV patients who were severely ill and those who were not willing to participate in the study were excluded from the study.

Study Design: 25 patients were selected under the category of suspected cases of HIV infection. High risk group patients, patients with any sign /

symptom suggestive of HIV infection are included in the list of suspected cases of HIV infection. High Risk group individuals include homosexual men, Intravenous drug users, Sex workers, Children born to HIV infected patients. Patients with General symptoms like Sudden weight loss, chronic fatigue and tiredness, Recurrent Fever and cough, atypical severe dermatological lesions, H/0 Tuberculosis and Oral symptoms such as Oral Candidiasis, Atypical Herpetic Lesions, Non healing Ulcers, Idiopathic severe burning sensation of Oral Mucosa, Severe Aggressive Gingivitis and periodontitis refractory to treatment are considered as clinically suspected cases of HIV infection. Selected cases are subjected to OMT Test and compared with Rapid Serum tests of antibody detection. Rapid Serum antibody detection test include 3 consecutive serum tests namely Type I COMBAIDS HIV 1+2 Immunodot assay, Type II SD BIOLINE HIV 1/ 2 Rapid serum assay, Type III Pareeshak HIV 1/2 Triline serum assay . The individuals who are tested positive to Type I Rapid serum assays are tested with other two tests and the results are read as positive to HIV infection only when the patient is positive to all the three Rapid serum assays. Pre and Posttest counseling was mandatory before the patients are subjected to HIV tests.

25 cases Of Known HIV patients are used as control group and are tested for Oral Mucosal Transudate test

Armamentarium:

- Diagnostic Instruments (Fig 1)
- Universal barriers: Disposable gloves, Masks, Protective goggles(Fig 3)
- Timer
- Test KIT : Aware HIV 1/2 OMT Kit consisting of (Fig 2)
    - Foil pouch containing Oral fluid test strip and desiccant.
    - Capped test tube containing oral fluid sample buffer
    - Oral fluid sample collection swab
- Biomedical Waste Disposal system (Fig 11-13)

Aware Oral Mucosal Transudate Test:

A qualitative, visually read, in vitro immunoassay for the detection of antibodies to Human Immunodeficiency Virus Type 1(HIV- 1) and Type 2 (HIV-2) in human oral mucosal transudate specimens. Aware HIV 1/2 OMT test is introduced by **Calytype Biomedical corporation, USA.** It is intended for use as appoint of care aid in the clinical diagnosis of HIV infection. The test may be used as a component of a multi- test rapid algorithm in conjunction with other approved HIV antibody assays. Individuals infected with HIV produce antibodies against the HIV viral proteins. Testing for the presence of these antibodies in bodily fluids (blood, urine, and oral fluid) is an accurate aid in diagnosis of infection. The Aware HIV 1/2 OMT test is composed of a single – use test device,

an oral fluid specimen, collection swab and a tube of oral fluid sample buffer. The test utilizes a proprietary lateral flow immunoassay procedure. The assay test strip is compromised of several materials that provide the matrix for the immunochromatography of the specimen. The assay strip contains recombinant regions of HIV -1 gp41 and HIV -2 gp 36 transmembrane proteins and a goat anti human IgG F (ab') 2 fragment antibody in- capture procedural control immobilized onto the nitrocellulose in the test zone and the control zone, respectively. The oral fluid swab is placed vertically into the test-tube of fluid buffer. The liquid in the swab is expressed out and the used swab is discarded. The assay strip is then placed vertically into the test tube containing the specimen / buffer mixture. As the diluted specimen migrates up the assay test strip, it rehydrates a reddish Protein A- colloidal gold reagent on the strip and IgG specimen becomes bound to the protein A / colloidal gold particles to form IgG / conjugate complex. The complex then migrates up the strip and first encounters the test zone of the assay strip containing the HIV antigens. If the specimen contains antibodies to HIV, IgG/conjugate complex binds to the antigen and becomes immolized at the antigen line in the test zone and a reddish colored line appears. In a validity performed test, this indicates a reactive result. The intensity of the line is not proportional to the amount of antibody present in the specimen. The Specimen/conjugate mixture continues to migrate up the assay strip until

it encounters the Control Zone. The Control Zone contains anti human IgG F (ab') 2 fragments immobilized in a line on the assay test strip. The complex bound to the immobilized F (ab') 2 fragments and a reddish colored line appears. The appearance of the control line is evidence that the test functioned properly and the sample contained human IgG. A reddish purple control line will appear in the control Zone during the performance of all valid tests, whether or not the sample is reactive or negative for antibodies to HIV 1/ 2. The specimen continues to migrate past the control Zone into the final absorbent pad, which helps draw the specimen / conjugate mixture through the strip and clear any background color. The Test results are interpreted after 20 minutes but not more than 45 minutes after introduction of the assay strip to the diluted specimen.

Procedure: Specimen collection (Fig 4-7):

Oral fluid sample collection swab is removed from the foil. Moderate pressure is applied while gently swabbing the upper gingiva back and forth with the cloth end of the swab. Beginning at one end of the mouth, swabbing is done gently and slowly until reaching the other corner of the mouth, covering the buccal mucosa. The other end of the swab is turned and is used for swabbing the lower gingiva. Immediately the swab is placed in the tube containing the sample buffer. The swab handle is grasped firmly and plunged up and down 6-8 times in the sample buffer rubbing both sides of the swab against the sides of the tube (Fig 8). The

swab is carefully removed and discarded in a biomedical waste container. Now the foil pouch is opened and the assay test strip is placed in the tube containing diluted specimen (fig 9) with arrow in the assay strip pointing downwards. Timer is set. The results are read after 20 minutes. The results are not read after 45 minutes.

Interpretation of Results (Fig 10)

The Results are interpreted as follows by appreciating the test line and control line in the test strip.

Reactive: The test is read as positive or reactive if Two lines appear in the assay strip (i.e.) in the test zone and control zone.

Non-reactive: The test is read as Negative or non reactive if only one line appear in the assay strip (i.e.) in the control zone only.

Invalid: The test is considered invalid if no control line is present. The invalid test should be repeated using a new test device and a second specimen.

Precautions:

Tests should be performed at ambient temperature 15-30 degrees. Universal precautions have to be taken care of when handling body fluids and assay strips. Patient asked not to drink, eat or smoke before the tests. Biomedical waste disposal system should be maintained to dispose the test specimens.

Profoma

Qualitative Immunoassay for HIV 1/2 Antibodies in Oral Mucosal Transudate -A Diagnostic Study

Department of Oral Medicine and Radiology

Tamilnadu Government Dental college and Hospital, Chennai- 600 003

Case no: **O.P. No:** **Date:**

1. Name:

2. Age: 3.Sex:

4. Address:

5. Occupation: 6.Income:

7. Martial Status:

8. Sample Category:

Known positive: Suspected case:

9. Clinical Examinations

1. Chief Complaint:

2. History of presenting illness:

3. Past Medical history:

S.no	Complaints	Yes/no	Duration
1	Weight loss		
2	Cough		
3	Fatigue		
4	Night sweats		
6	Fever		
7	Lymphadenopathy		

Specific Systems:

S.no	Systems	Yes/No	Symptoms	Duration
1	Respiratory system			
2	Gastro intestinal system			
3	Central nervous system			
4	Cardio Vascular system			
5	Dermatological lesions			
6	Psychological Status			

4. General Examinations:

i) Weight: ii) Height:

iii) Nutritional Status: Good / Moderate / Poor

iv) Built: Well built/ moderate/ Poor

v) Vital Signs:

Blood pressure: Respiratory rate:

Pulse Rate: Temperature:

vi) Constitutional signs:

Anemia: Cyanosis:

Clubbing: Jaundice:

vii) Lymph node Examinations:

Group:

Tenderness:

Number: Size: Consistency:

4. HIV Positive cases:

Contact history:

a) Heterosexual
b) Homosexual
c) Vertical transmission
d) Commercial Sex Worker
e) Intravenous drug abuser
f) Spouse

Local Examination:

Extra oral examination:

Intra oral examination:

Soft Tissue Examination:

Upper Lip:

Lower Lip:

Commissures:

Buccal Mucosa:

Tongue:

Floor of mouth:

Alveolar Mucosa:

Gingiva:

Hard palate:

Soft palate:

Hard Soft tissue Examination:

No of teeth: Decayed tooth:

Attrition: Abrasion:

Erosion: Stains:

Investigations:

Routine Blood investigations:

TC: DC: ESR: Hb%:

BT: CT: RBC count:

Peripheral smear:

Sputum for AFB:

Chest x-ray:

Other investigations:

Treatment:

Oral Mucosal transudate test (OMT test)

Region of interest: (Region where swab taken):

Positive:

Negative:

Known positive patients:

Duration of illness:

Type of test done:

ART (Anti retro viral therapy): YES / NO

Duration of ART:

Suspected cases:

Type of test done:

Positive:

Negative:

PATIENT INFORMED CONSENT FORM

Name: Age:

OP.No: Sex:

I …… Aged …….. years is informed about the HIV diseases and about the study to test HIV infection in oral fluid. I hereby give my consent to be included as a participant in this diagnostic study.

Patients Signature:

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Results and Analysis

Tables and Charts

Age: Table 1

Group Statistics

	GROUP	N	Mean	Std. Deviation	Std. Error Mean
Age	Known Positive	25	36.40	11.113	2.223
	Suspected Cases	25	37.24	10.910	2.182

Sex: Table 2

Crosstab

			GROUP		
			Known Positive	Suspected Cases	Total
Sex	Male	Count	18	17	35
		% within GROUP	72.0%	68.0%	70.0%
	Female	Count	7	8	15
		% within GROUP	28.0%	32.0%	30.0%
Total		Count	25	25	50
		% within GROUP	100.0%	100.0%	100.0%

Place: Table 3

Crosstab

			GROUP		
			Known Positive	Suspected Cases	Total
Place	Chennai	Count	19	22	41
		% within GROUP	76.0%	88.0%	82.0%
	Out side chennai	Count	6	3	9
		% within GROUP	24.0%	12.0%	18.0%
Total		Count	25	25	50
		% within GROUP	100.0%	100.0%	100.0%

Contact history: (Known Positive) Table 4

Crosstab

			GROUP	
			Known Positive	Total
Contact History	Heterosexual	Count	16	16
		% within GROUP	64.0%	64.0%
	Vertical transmission	Count	5	5
		% within GROUP	20.0%	20.0%
	Spouse	Count	3	3
		% within GROUP	12.0%	12.0%
	Intravenous drug User	Count	1	1
		% within GROUP	4.0%	4.0%
Total		Count	25	25
		% within GROUP	100.0%	100.0%

Under ART: (Known Positive) Table 5

Crosstab

			GROUP		
			Known Positive	Suspected Cases	Total
Under ART/ Not	Yes	Count	11	0	11
		% within GROUP	44.0%	.0%	22.0%
	No	Count	14	25	39
		% within GROUP	56.0%	100.0%	78.0%
Total		Count	25	25	50
		% within GROUP	100.0%	100.0%	100.0%

Chronic Cough: Table 6

Crosstab

			GROUP		
			Known Positive	Suspected Cases	Total
Chronic cough	Yes	Count	4	8	12
		% within GROUP	16.0%	32.0%	24.0%
	No	Count	21	17	38
		% within GROUP	84.0%	68.0%	76.0%
Total		Count	25	25	50
		% within GROUP	100.0%	100.0%	100.0%

Recurrent Fever: Table 7

Crosstab

			GROUP		
			Known Positive	Suspected Cases	Total
Recurrent Fever	Yes	Count	2	6	8
		% within GROUP	8.0%	24.0%	16.0%
	No	Count	23	19	42
		% within GROUP	92.0%	76.0%	84.0%
Total		Count	25	25	50
		% within GROUP	100.0%	100.0%	100.0%

Weight Loss: Table 8

Crosstab

			GROUP		
			Known Positive	Suspected Cases	Total
Weight Loss	Yes	Count	6	14	20
		% within GROUP	24.0%	56.0%	40.0%
	No	Count	19	11	30
		% within GROUP	76.0%	44.0%	60.0%
Total		Count	25	25	50
		% within GROUP	100.0%	100.0%	100.0%

Fatigue/Tiredness: Table 9

Crosstab

			GROUP		
			Known Positive	Suspected Cases	Total
fatigue/tiredness	Yes	Count	3	8	11
		% within GROUP	12.0%	32.0%	22.0%
	No	Count	22	17	39
		% within GROUP	88.0%	68.0%	78.0%
Total		Count	25	25	50
		% within GROUP	100.0%	100.0%	100.0%

H/O TB: Table 10

Crosstab

			GROUP		
			Known Positive	Suspected Cases	Total
H/o TB	Yes	Count	2	2	4
		% within GROUP	8.0%	8.0%	8.0%
	No	Count	23	23	46
		% within GROUP	92.0%	92.0%	92.0%
Total		Count	25	25	50
		% within GROUP	100.0%	100.0%	100.0%

Others: Table 11

Crosstab

			GROUP		
			Known Positive	Suspected Cases	Total
Others	Yes	Count	1	7	8
		% within GROUP	4.0%	28.0%	16.0%
	No	Count	24	18	42
		% within GROUP	96.0%	72.0%	84.0%
Total		Count	25	25	50
		% within GROUP	100.0%	100.0%	100.0%

Oral Manifestations:

Pseudomemebranous GROUP: Table 12

Crosstab

			GROUP		
			Known Positive	Suspected Cases	Total
Pseudomemebranous	Yes	Count	2	4	6
		% within GROUP	8.0%	16.0%	12.0%
	No	Count	23	21	44
		% within GROUP	92.0%	84.0%	88.0%
Total		Count	25	25	50
		% within GROUP	100.0%	100.0%	100.0%

Erythematous GROUP: Table 13

Crosstab

			GROUP		
			Known Positive	Suspected Cases	Total
Erythematous	Yes	Count	5	10	15
		% within GROUP	20.0%	40.0%	30.0%
	No	Count	20	15	35
		% within GROUP	80.0%	60.0%	70.0%
Total		Count	25	25	50
		% within GROUP	100.0%	100.0%	100.0%

Angular chelitis GROUP: Table 14

Crosstab

			GROUP		
			Known Positive	Suspected Cases	Total
Angular chelitis	Yes	Count	2	5	7
		% within GROUP	8.0%	20.0%	14.0%
	No	Count	23	20	43
		% within GROUP	92.0%	80.0%	86.0%
Total		Count	25	25	50
		% within GROUP	100.0%	100.0%	100.0%

Non healing Ulcers: Table 15

Crosstab

			GROUP		
			Known Positive	Suspected Cases	Total
Non healing Ulcers	Yes	Count	3	3	6
		% within GROUP	12.0%	12.0%	12.0%
	No	Count	22	22	44
		% within GROUP	88.0%	88.0%	88.0%
Total		Count	25	25	50
		% within GROUP	100.0%	100.0%	100.0%

Recurrent Apthous Ulcers: Table 16

Crosstab

			GROUP		
			Known Positive	Suspected Cases	Total
Recurrent Apthous Ulcers	Yes	Count	1	2	3
		% within GROUP	4.0%	8.0%	6.0%
	No	Count	24	23	47
		% within GROUP	96.0%	92.0%	94.0%
Total		Count	25	25	50
		% within GROUP	100.0%	100.0%	100.0%

Malignancies GROUP: Table 17

Crosstab

			GROUP		
			Known Positive	Suspected Cases	Total
Malignancies	Yes	Count	2	1	3
		% within GROUP	8.0%	4.0%	6.0%
	No	Count	23	24	47
		% within GROUP	92.0%	96.0%	94.0%
Total		Count	25	25	50
		% within GROUP	100.0%	100.0%	100.0%

Salivary gland enlargements: Table 18

Crosstab

			GROUP	
			Known Positive	Total
Salivary gland enlargements	No	Count	25	25
		% within GROUP	100.0%	100.0%
Total		Count	25	25
		% within GROUP	100.0%	100.0%

Xerostomia: Table 19

Crosstab

			GROUP	
			Known Positive	Total
Xerostomia	Yes	Count	2	2
		% within GROUP	8.0%	8.0%
	No	Count	23	23
		% within GROUP	92.0%	92.0%
Total		Count	25	25
		% within GROUP	100.0%	100.0%

Periodontitis: Table 20

Crosstab

			GROUP		
			Known Positive	Suspected Cases	Total
Periodontitis	Yes	Count	9	11	20
		% within GROUP	36.0%	44.0%	40.0%
	No	Count	16	14	30
		% within GROUP	64.0%	56.0%	60.0%
Total		Count	25	25	50
		% within GROUP	100.0%	100.0%	100.0%

Pigmentations: Table 21

Crosstab

			GROUP	
			Known Positive	Total
Pigmentations	Yes	Count	5	5
		% within GROUP	20.0%	20.0%
	No	Count	20	20
		% within GROUP	80.0%	80.0%
Total		Count	25	25
		% within GROUP	100.0%	100.0%

Others: Table 22

Crosstab

			GROUP		
			Known Positive	Suspected Cases	Total
Others	Yes	Count	2	10	12
		% within GROUP	8.0%	40.0%	24.0%
	No	Count	23	15	38
		% within GROUP	92.0%	60.0%	76.0%
Total		Count	25	25	50
		% within GROUP	100.0%	100.0%	100.0%

OMT GROUP: Table 23

Crosstab

			GROUP		
			Known Positive	Suspected Cases	Total
OMT	Positive	Count	23	8	31
		% within GROUP	92.0%	32.0%	62.0%
	Negative	Count	2	17	19
		% within GROUP	8.0%	68.0%	38.0%
Total		Count	25	25	50
		% within GROUP	100.0%	100.0%	100.0%

Chronic cough * Under ART/ Not: Table 24

Crosstab

			Under ART/ Not		
			Yes	No	Total
Chronic cough	Yes	Count	2	2	4
		% of Total	8.0%	8.0%	16.0%
	No	Count	9	12	21
		% of Total	36.0%	48.0%	84.0%
Total		Count	11	14	25
		% of Total	44.0%	56.0%	100.0%

Recurrent Fever * Under ART/ Not: Table 25

Crosstab

			Under ART/ Not		
			Yes	No	Total
Recurrent Fever	Yes	Count	2	0	2
		% of Total	8.0%	.0%	8.0%
	No	Count	9	14	23
		% of Total	36.0%	56.0%	92.0%
Total		Count	11	14	25
		% of Total	44.0%	56.0%	100.0%

Weight Loss * Under ART/ Not: Table 26

Crosstab

			Under ART/ Not		
			Yes	No	Total
Weight Loss	Yes	Count	2	4	6
		% of Total	8.0%	16.0%	24.0%
	No	Count	9	10	19
		% of Total	36.0%	40.0%	76.0%
Total		Count	11	14	25
		% of Total	44.0%	56.0%	100.0%

Fatigue/tiredness * Under ART/ Not: Table 27

Crosstab

			Under ART/ Not		
			Yes	No	Total
fatigue/tiredness	Yes	Count	2	1	3
		% of Total	8.0%	4.0%	12.0%
	No	Count	9	13	22
		% of Total	36.0%	52.0%	88.0%
Total		Count	11	14	25
		% of Total	44.0%	56.0%	100.0%

H/o TB * Under ART/ Not: Table 28

Crosstab

			Under ART/ Not		
			Yes	No	Total
H/o TB	Yes	Count	1	1	2
		% of Total	4.0%	4.0%	8.0%
	No	Count	10	13	23
		% of Total	40.0%	52.0%	92.0%
Total		Count	11	14	25
		% of Total	44.0%	56.0%	100.0%

Others * Under ART/ Not: Table 29

Crosstab

			Under ART/ Not		
			Yes	No	Total
Others	Yes	Count	0	1	1
		% of Total	.0%	4.0%	4.0%
	No	Count	11	13	24
		% of Total	44.0%	52.0%	96.0%
Total		Count	11	14	25
		% of Total	44.0%	56.0%	100.0%

Pseudomemebranous * Under ART/ Not: Table 30

Crosstab

			Under ART/ Not		
			Yes	No	Total
Pseudomemebranou	Yes	Count	0	2	2
		% of Total	.0%	8.0%	8.0%
	No	Count	11	12	23
		% of Total	44.0%	48.0%	92.0%
Total		Count	11	14	25
		% of Total	44.0%	56.0%	100.0%

Erythematous * Under ART/ Not: Table 31

Crosstab

			Under ART/ Not		
			Yes	No	Total
Erythematou	Yes	Count	1	4	5
		% of Tota	4.0%	16.0%	20.0%
	No	Count	10	10	20
		% of Tota	40.0%	40.0%	80.0%
Total		Count	11	14	25
		% of Tota	44.0%	56.0%	100.0%

Angular chelitis * Under ART/ Not: Table 32

Crosstab

			Under ART/ Not		
			Yes	No	Total
Angular chelitis	Yes	Count	0	2	2
		% of Total	.0%	8.0%	8.0%
	No	Count	11	12	23
		% of Total	44.0%	48.0%	92.0%
Total		Count	11	14	25
		% of Total	44.0%	56.0%	100.0%

Non healing Ulcers * Under ART/ Not: Table 33

Crosstab

			Under ART/ Not		
			Yes	No	Total
Non healing Ulcers	Yes	Count	1	2	3
		% of Total	4.0%	8.0%	12.0%
	No	Count	10	12	22
		% of Total	40.0%	48.0%	88.0%
Total		Count	11	14	25
		% of Total	44.0%	56.0%	100.0%

Recurrent Apthous Ulcers * Under ART/ Not: Table 34

Crosstab

			Under ART/ Not		
			Yes	No	Total
Recurrent Apthous Ulcers	Yes	Count	0	1	1
		% of Total	.0%	4.0%	4.0%
	No	Count	11	13	24
		% of Total	44.0%	52.0%	96.0%
Total		Count	11	14	25
		% of Total	44.0%	56.0%	100.0%

Malignancies * Under ART/ Not: Table 35

Crosstab

			Under ART/ Not		
			Yes	No	Total
Malignancies	Yes	Count	1	1	2
		% of Total	4.0%	4.0%	8.0%
	No	Count	10	13	23
		% of Total	40.0%	52.0%	92.0%
Total		Count	11	14	25
		% of Total	44.0%	56.0%	100.0%

Salivary gland enlargements * Under ART/ Not: Table 36

Crosstab

			Under ART/ Not		
			Yes	No	Total
Salivary gland enlargements	No	Count	11	14	25
		% of Total	44.0%	56.0%	100.0%
Total		Count	11	14	25
		% of Total	44.0%	56.0%	100.0%

Xerostomia * Under ART/ Not: Table 37

Crosstab

			Under ART/ Not		
			Yes	No	Total
Xerostomia	Yes	Count	1	1	2
		% of Total	4.0%	4.0%	8.0%
	No	Count	10	13	23
		% of Total	40.0%	52.0%	92.0%
Total		Count	11	14	25
		% of Total	44.0%	56.0%	100.0%

Periodontitis * Under ART/ Not: Table 38

Crosstab

			Under ART/ Not		
			Yes	No	Total
Periodontitis	Yes	Count	4	5	9
		% of Total	16.0%	20.0%	36.0%
	No	Count	7	9	16
		% of Total	28.0%	36.0%	64.0%
Total		Count	11	14	25
		% of Total	44.0%	56.0%	100.0%

Pigmentations * Under ART/ Not: Table 39

Crosstab

			Under ART/ Not		
			Yes	No	Total
Pigmentations	Yes	Count	2	3	5
		% of Total	8.0%	12.0%	20.0%
	No	Count	9	11	20
		% of Total	36.0%	44.0%	80.0%
Total		Count	11	14	25
		% of Total	44.0%	56.0%	100.0%

Others * Under ART/ Not: Table 40

Crosstab

			Under ART/ Not		
			Yes	No	Total
Others	Yes	Count	0	2	2
		% of Total	.0%	8.0%	8.0%
	No	Count	11	12	23
		% of Total	44.0%	48.0%	92.0%
Total		Count	11	14	25
		% of Total	44.0%	56.0%	100.0%

Sensitivity and Specificity:

Table: 41

OMT * Serum assay Crosstabulation

			Serum assay		
			Positive	Negative	Total
OMT	Positive	Count	31	0	31
		% within Serum assay	93.9%	.0%	62.0%
	Negative	Count	2	17	19
		% within Serum assay	6.1%	100.0%	38.0%
Total		Count	33	17	50
		% within Serum assay	100.0%	100.0%	100.0%

Sensitivity of OMT Test: (31/33) × 100 = 93.93 %
The percentage of the results that will be positive when HIV is present.

Specificity of OMT Test: 100%.
The Percentage of the results that will be negative when HIV is not present

Positive Predictive Value: 100%

Negative Predictive Value: 89.4%

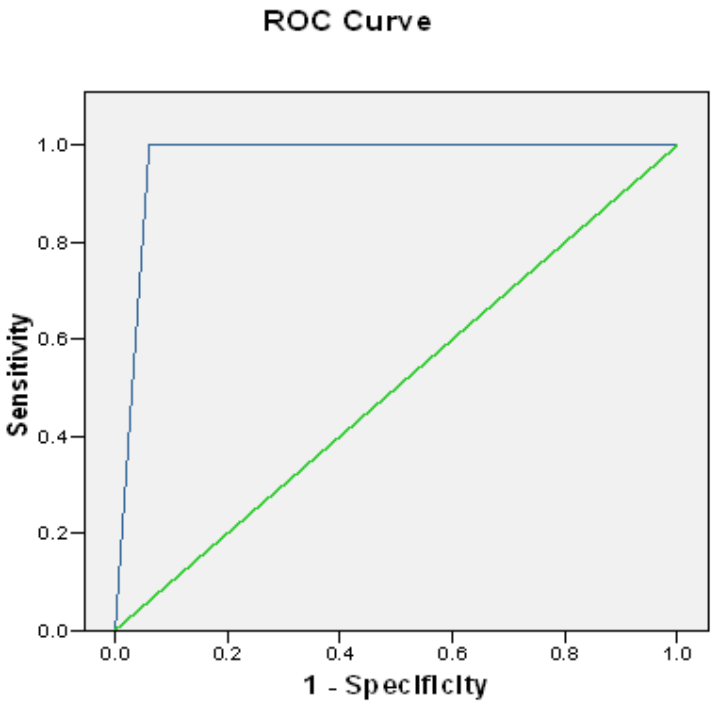
ROC Curve: (Receiver Operative Characteristics Curve) Graph1

Case Processing Summary

Serum assay	Valid N (listwise)
Positive[a]	17
Negative	33

Larger values of the test result variable(s) indicate stronger evidence for a positive actual state.

a. The positive actual state is Negative.



Diagonal segments are produced by ties.

Area Under the Curve

Test Result Variable(s): OMT

Area
.970

The test result variable(s): OMT has at least one tie between the positive actual state group and the negative actual state group.

Results and analysis

This study is evaluated to test the sensitivity, specificity, positive predictive value and negative predictive value of Calypte's AWARE^{TM} Oral mucosal transudate rapid test for detecting antibodies to HIV 1/ 2 in oral fluid and is compared with standard serum rapid test approved by National AIDS control Organization. Sample sizes of 50 patients were selected and grouped in two groups namely Known Sero-positive (Group1) and suspected cases of HIV (Group 2). Group 1 includes 25 cases (50%) of HIV Known Sero-positive HIV infected individuals who came to the dept of Oral Medicine and Radiology for various dental treatments. Group 2 includes 25 cases (50%) of clinically suspected cases of HIV infection. All patients were under age group 20- 60yrs. Out of 50 patients the mean age group of Group 1 were 36 and Group 2 was 37(Table 1). Out of which 35 were males 15 were females (Table 2), 41 belonging to Chennai and 9 were outsiders (Table 3). With their informed consent and Pre counseling Oral Mucosal transudate test were carried out and the results were analyzed using SPSS software. Of Known HIV Sero-positive individuals the contact history revealed 64% acquired infection through heterosexual route, 20% through vertical transmission, 12% through spouse, and 4% through intravenous drug usage with 22% under ART (Table 4).

On examining, general manifestations of the individuals of both groups, 24% were with chronic cough(Table 6), 16% with recurrent fever(Table 7), 40% with weight loss(Table 8), 22 % with fatigue and tiredness(Table 9), 8% with previous / present attack of tuberculosis(Table 10) and 16% with other general manifestations(Table 11). Analyzing the presence of oral manifestations in the samples selected, 12% were affected with pseudo membranous candidiasis (Table 12), 15% with erythematous candidiasis (Table 13), 14% with angular chelitis (Table 14), 12% with non healing ulcers (Table 15), 6% with recurrent apthous ulcers (Table 16), 20% with periodontal related lesions (gingivitis, periodontitis, necrotizing gingivitis and periodontitis) (Table 20) and 12% with other oral manifestations (Table 22). In Known positive group 8% were affected with xerostomia (Table 19) and 20% were having mucosal pigmentations (Table 21). Known Positive group (group1) were divided into two groups namely the persons who were taking ART and persons who were not under ART treatment. Out of 25 cases of Group 1 11(44%) patients were under ART and 14 (56%) were not under ART. Analyzing the samples under ART of Known positive patients with Non ART group, 8% were affected with cough (Table 24) in both ART group and non-ART group, 2patients(8%) with recurrent fever in ART group whereas none was affected in not under ART group (Table 25), 8% with weight loss in ART group whereas 16% were affected in not under ART group

(Table 26), 8% with fatigue and tiredness in ART group whereas only 4% in not under ART group (Table 27), and 4 % with history of attack of tuberculosis in both ART and not under ART group (Table 28) which was significantly lower than patients under non ART group. Comparing the oral manifestations of patients with ART and patients not under ART of known positive patients, people with pseudo membranous candidiasis under ART group was 0% whereas patients with not under ART group was 8%(Table 30). Patients suffering from Erythematous candidiasis in ART group were 4% and in not under ART group was 16 % (Table 31). Angular chelitis was 0% in ART group and 8% in patients not under ART group (Table 32), non healing ulcers was 4% in ART group and 8% in patients not under ART group (Table 33), Recurrent Apthous ulcers were 0% in ART group and 4% in patients not under ART group (Table 34), salivary enlargements were 44% in ART group (Table 35) and 56% in patients not under ART group , periodontitis were 16 in ART group and 20% in patients not under ART group(Table 38), pigmentations were 8% in ART group and 12% in patients not under ART group(Table 39). The data clearly shows that there is a decrease in both general and oral manifestations in patients under ART group. Analyzing the results of oral mucosal transudate test, Out of 25 patients of Known Seropositive group 23 showed positive results in OMT test; where as 2 patients were negative to OMT test. In Group 2 of Suspected cases of HIV individuals

8 patients (32%) were positive to OMT test and 17 patients (68%) were negative to OMT test (Table 41). Group 2 patients either positive or negative to OMT test were subjected to Standard Rapid Serum assays to test HIV infection and the results were compared with OMT test results. 17 patients who were negative to OMT test were also negative to Rapid serum assay and 8 patients who were positive to OMT test were also positive to Rapid serum assay (Table 41). From the statistical analysis the sensitivity of the OMT rapid screening assay was 93.93% and specificity was 100%. The positive predictive value was computed to be 100% with a negative predictive value of 87.4%. Receptor Operator characteristic curve (Graph 1) which is plotted against sensitivity and specificity of OMT Test clearly shows the area under the curve is 0.970 indicating that OMT test is better useful as a screening test.

Discussion

The main aim of this study is to evaluate the efficacy of Oral Fluid in diagnosing and screening individuals with HIV infection and to compare the Sensitivity and Specificity of Oral Mucosal transudate test with standard serum screening test. Epidemiological studies need easier tools to evaluate HIV prevalence particularly in high risk groups under difficult field conditions. Testing Saliva/ Oral mucosal antibody with accurate immunoassay could serve as an alternative to serum assays. Salivary assays could be efficient and non invasive epidemiological tool for HIV testing.

Whole saliva is a mixture of oral fluids and includes secretions from both minor and major salivary glands , in addition to several constituents of non-salivary origin such as gingival crevicular fluid (GCF), expectorated bronchial and nasal secretions , serum and blood derivatives from oral wounds, bacteria and bacterial products, viruses and fungi, desquamated epithelial cells, other cellular components and food debris. There are several ways by which the serum constituents that are not part of normal salivary constituents can reach saliva. A serum molecule reaching saliva by diffusion must cross five barriers; the capillary wall, interstitial space , basal cell membrane of the acinus cell/duct cell, cytoplasm of the duct cell, luminal cell membrane. Serum

constitituents are also found in whole saliva as a result of GCF outflow **(Eliaz Kaufman, Ira B. Lamster 2002)[10].** Depending on the degree of inflammation in gingival, GCF is a serum transudate or an inflammatory exudate that contains serum constituents. This Study aims at detection of IgG anti HIV antibody in oral fluid and hence called as **Oral Mucosal Transudate test.** The antigens to HIV-1(gp 41) and HIV-2 (gp 36) were impregnated in the assay strip to test the presence of HIV antibody to either HIV type 1 virus or Type 2 virus. So this test qualitatively analyses the presence of antibodies to HIV infection of both type 1 and type 2. In this study sample size of 50 cases were selected and divided in two groups namely Known Sero-Positive patients and Suspected cases of HIV infection. Oral examination is an important part of any physical examination and nowhere is this more important than in the case of suspected HIV infection **(Greenspan.D 1993)[76]**. As by saying "Mouth is a mirror of systemic diseases" oral manifestations of HIV affected individuals are typical and in most cases be an indicator of poor immunosuppression and HIV infection. Suspected cases were selected accordingly by the presence of any one or more of the following conditions namely

High Risk group individual

Homosexuals

Extramarital Sex

Intravenous drug users

Sex Workers

Children of HIV infected individuals

Occupationally Risk groups

General manifestations:

Chronic Cough for more than 6 months

Recurrent Fever for more than 6 months

Sudden Weight loss of about 10kgs

Fatigue/ Tiredness

H/O tuberculosis

Others

- Severe Dermatological Lesions
- Malignancies
- Respiratory and others serious infections

Oral manifestations:

Oral candidiasis

- Psedomembranous
- Atrophic/ erythemaotus
- Angular Chelitis

Non Healing Ulcers

Recurrent Apthous Ulcers.

Severe Periodontits/ Gingivitis with necrosis.

Others

- Malignancies

According to this study patients under ART group showed few general and oral manifestations of the HIV infection comparing with Patients under Non ART group. Also HIV positive patients tested under suspected cases of HIV infection were presented with increased severity of oral manifestations than Known Seropositive HIV individuals. From these results it is evident that oral manifestations of HIV infections are more common in untreated, unknown cases of HIV individuals than with patients under HAART treatment and regular follow up of HIV infection. Oral Manifestations and other systemic manifestations subsequently reduced after starting treatment with antiretroviral drugs. However side effects of HAART including xerostomia, mucosal pigmentations and salivary gland enlargements increase in frequency of patients under ART. According to this study the sensitivity of Oral Mucosal Transudate test is 93.93 %, specificity is 100%, the positive predictive value is 100% and negative predictive value is 89.4%. Receptor Operator characteristic curve of this study plotted against the sensitivity and specificity showed and area of 0.90 under the curve which clearly indicated that this test can be used as a Screening test to diagnose HIV infection. The Dental clinicians are more prone to HIV cross infections and it is necessary for the dental clinicians to cross check individuals affected with HIV infection to prevent HIV cross infection. All staffs, working personnel, dental surgeons and patients have to be protected against cross infections

of HIV. Oral Mucosal transudate test , an easy, simple , non invasive method for screening HIV infection would be a valuable tool and a chair side diagnostic aid for Oral medicine Specialist to screen patients undergoing dental treatment and for those clinically suspected cases of HIV infection. Application of Oral Fluid as a diagnostic aid for HIV infection would bring a remarkable change in dentistry[35] and also it would be a better screening tool for epidemiological studies and screening for HIV infection in high risk group individuals[35]. In this study the specificity of HIV infection is 100% indicating that this test is a valid screening test. However large sample size and further longitudinal studies is necessary to use this test as a screening test and validating this test as standard test for HIV infection.

Dental Considerations in HIV patients

Oral health considerations for persons infected with HIV focus on provision of dental care and oral conditions associated with their underlying disease. An appropriate work-up for an HIV infected patient needs to ascertain a patient overall health immune status , prognosis , presence and history of opportunistic infections, risk for developing more severe opportunistic infections and oral lesions, current medications and chance for long term survival. As a general rule, no dental modifications

are required for patient based on their HIV status[4,70]. Most individuals presenting to Out-patient dental care are sufficiently healthy to tolerate all types of dental procedures, ranging from scaling to implants. The major concerns are impaired homeostasis, susceptibility to dentally induced infections, drug interactions and the patient ability to withstand stress and trauma of dental procedures. Even when patients CD4 count are very low they are not more susceptible to dentally induced bacteraemia. There are no indications for routine use of antibiotic prophylaxis based on patients HIV status. However patients with neutrophil count below 500-700 cells /mm^3 require antibiotic[1]. Furthermore some patients maybe at a risk for developing sub acute bacterial endocarditis if they are former intravenous drug user. Most side effects from medications used to treat HIV disease are associated with Xerostomia. Drug interactions with medications commonly used in a dental setting also exist. Treatment planning for HIV positive patients needs to address numerous considerations. The vast majority of patients have some degree of Xerostomia ranging from mild to severe. Thus when performing simple restorative procedures or fabricating fixed / removable prosthodontics, the type of restorative material, long term use and maintenance of a restoration need to be taken into considerations[70]

Summary and Conclusion

HIV rapid testing devices are easy to use, do not require refrigeration and are less expensive to run, portable with a longer shelf life. They are more adaptable for field use and screening of high risk and far to reach populations mostly located in resource poor settings[14,35,37]. Infact, they have reduced the time between testing and release of results to minutes from days or weeks when compared with other assays. The need for regularly updating diagnostic principles and methods & continuous evaluation of test kits to make sure that they can detect the newly emerging viral strains is important. HIV 1/ 2 OMT evaluation result falls within the WHO recommended range of tests to be used for HIV diagnosis.

In this Study sensitivity and specificity of HIV 1/ 2 OMT test is evaluated in a sample size of 50 patients divided in two groups namely, known Sero-positive and suspected cases of HIV infection. The Sensitivity of the test is 93.93%, specificity is 100% with positive predictive value corresponds to 100% and negative predictive value corresponds to 89.4%. From the results it's very clear that Oral mucosal transudate test can be used as a screening test for large scale high risk group individuals in a community, suspected patients in dental health care settings. Though a confirmatory testing is required using a standard

serum test before disclosing positive result to a patient. Cross infections in a dental community or health care workers can be readily minimized when Oral mucosal transudate test is used in dental care patients. CDC continues to encourage the use of rapid HIV tests[20-22,35] because they increase the number of persons who are tested and who receive their tests results. Regardless confirmatory testing is required to confirm, all patients should be informed that reactive rapid oral fluid tests are preliminary and require confirmation[35]. Overall, Oral fluid rapid tests have performed well and make HIV testing possible in many venues where performing serum tests are impractical for screening. The main advantage of rapid HIV oral fluid tests includes increased availability and acceptability of testing among populations at high risk for HIV infection and is simple, easy, non-invasive, user friendly[52] and prevents cross infection to health care workers. From this study we conclude that Oral fluid can be used as an efficient Screening tool for diagnosis of HIV infection[35]. Further longitudinal studies, increased sample size is required to test the efficacy of Oral Fluid / Saliva in diagnosing HIV infection and using OMT test as a screening test in practice.

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Photographs
Armamentarium

Diagnostic Instruments
Fig 1

Diagnostic Kit
Fig 2

Universal barriers
Fig 3

Procedure

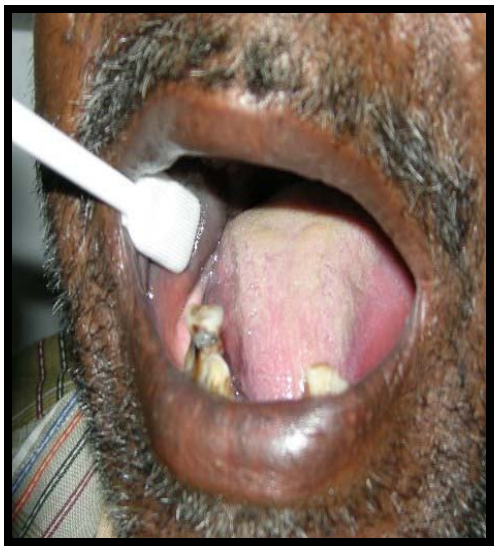
Step 1

Fig 4

Fig 5

Fig 6

Fig 7



Oral Mucosal fluid swab is taken from

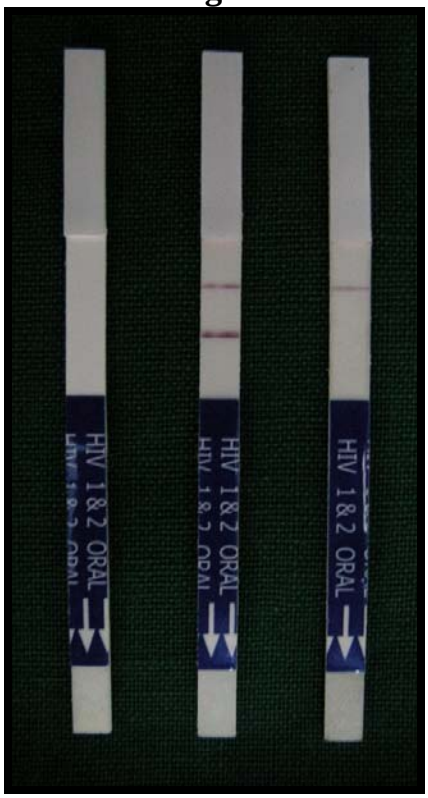
Gingiva and Buccal mucosa

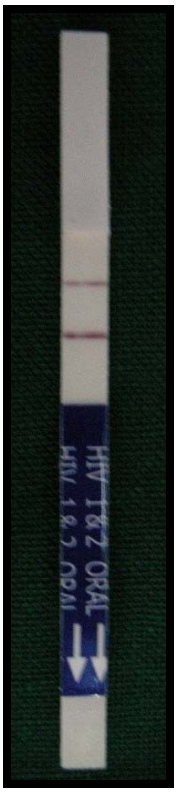
Step 2 (Fig 8) **Step 3 (Fig 9)**

Oral mucosal swab is mixed into the buffer Test strip is inserted into the buffer

Interpretation of Results

Fig 10





Biomedical Waste Management

Step 1(Fig 11)

1) Outer Wrappers disposed into the Green Colored bin

Step 2 (Fig 12)

Step 3 (Fig 13)

2) Swab, buffer, test strip placed in biohazard cover and 10% sodium hypochlorite is added and 3) disposed into the Twin red colored bin.

Clinical Cases- Oral manifestations of HIV

Case 1

Multiple Non Healing Ulcers

Case 2

Multiple Recurrent Apthous Ulcer

Case 3

Chronic atrophic candidiasis

Case 4

Chronic atropic candidiasis

Pseudomembranous candidiasis

Case 5

Chronic atropic candidiasis

Case 6

HIV induced Melanosis of palate

Case 7

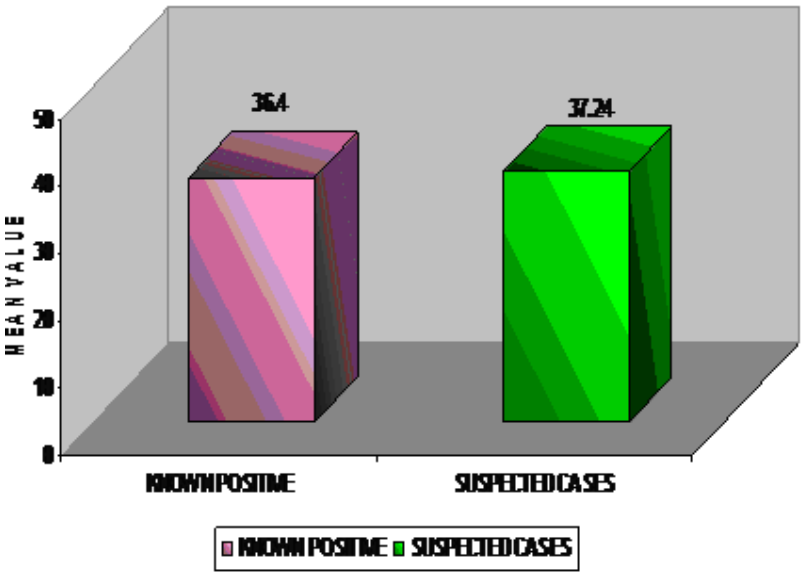
OSMF with Leukoplakia

Case 8

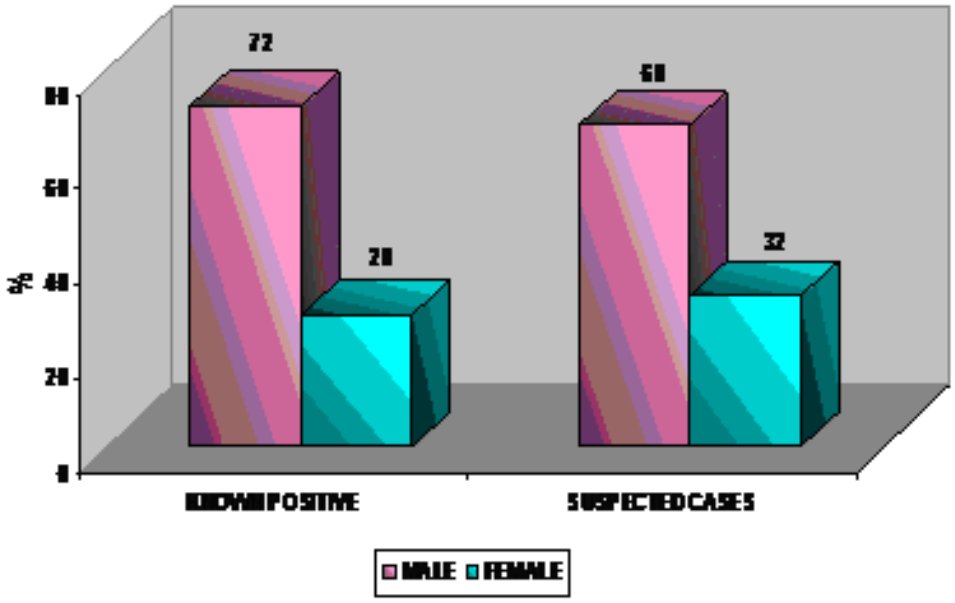
Non Hodgkins Lymphoma

Charts

Age Vs Known Sero-Positive/ Suspected case

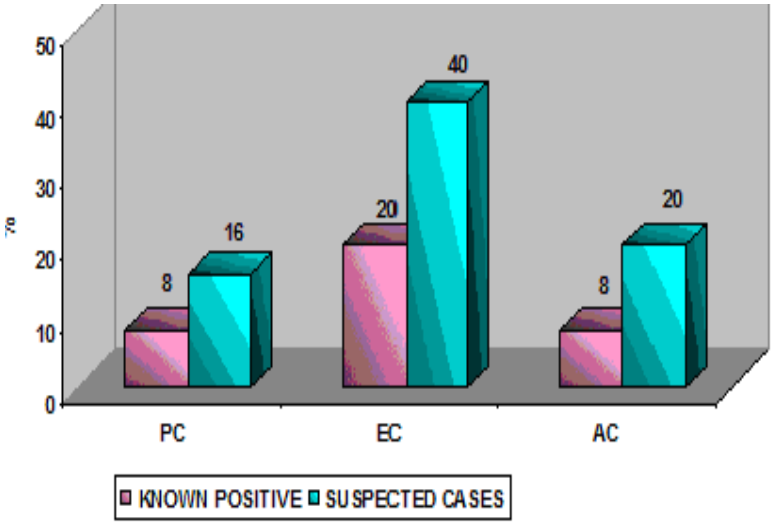


Sex VS Known Sero-Positive / Suspected cases



Oral Candidiasis Vs Known Sero-Positive /Suspected cases

PC –Psedomembranous candidiasis EC- Erythematous candidiasis
AC- Angular Chelitis



Other Oral Manifestations VS Known Sero-Positive/ suspected cases

NHU- Non Healing ulcers, RU- Recurrent ulcers, M- Malignancies (lymphoma, Squamous cell carcinoma)
Perio- Periodontal lesions, Pig- Pigmentations